Negatively-charged lipid membranes have been suggested to trigger ''amyloid-like'' fibril formation by several non-amyloidogenic proteins, *e.g.* lysozyme [1]. We aimed to elucidate the factors that govern the formation of these ''amyloid-like'' fibrils and to characterize their structural and dynamical properties. Lysozyme was labeled with Alexa 488 (A488-Lz) and its interaction with POPC LUVs containing 20 and 30 mol% of POPS was studied using both steady-state and time-resolved fluorescence techniques. The variation of the mean fluorescence lifetime of A488-Lz as a function of the surface coverage of the liposomes was quantitatively described by a three-state model that assumes that monomeric lysozyme molecules partition into the bilayer surface and reversibly assemble into oligomers with $N$ subunits ($N \geq 6$) (cooperative partition model). The global fit was done using the partition coefficients previously determined for A488-Lz by fluorescence correlation spectroscopy (FCS) [2] and by taking into account electrostatic effects by means of the Gouy-Chapman theory. To better evaluate the oligomerization state of membrane-bound lysozyme, the steady-state fluorescence anisotropy of A488-Lz was also measured for two different fluorophore labeling. The extent of energy migration between A488-Lz (decrease in fluorescence anisotropy) was adequately described only for $N = 6 \pm 1$ when the binomial distribution of fluorescently-labeled monomers among the oligomers was considered. Finally, the lipid-protein supramolecular complexes formed at a low lipid/protein molar ratio [1] were characterized by fluorescence lifetime imaging microscopy (FLIM). The average fluorescence lifetime of A488-Lz had a uniform spatial distribution on these structures, being much shorter than the values measured for free and membrane-bound monomeric A488-Lz, reporting the aggregated state of lysozyme.

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[2] Melo *et al*. **2011** *Biochim. Biophys. Acta* 1808: 2559

**2203-Plat**

**Effects of Lipids on the Conformation and Aggregation of the Repeat Domain of a Functional Amyloid, Pmel17**

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Pmel17 is a structural protein involved in melanin synthesis and deposition that forms fibrous striations in melanosomes, acidic organelles where pigmentation occurs. It was recently shown that a fragment of Pmel17 named the repeat domain (RPT, residues 315-444) is responsible for fibril formation in vitro under mildly acidic conditions. (McGlinchey RP. et al., PNAS, 2009; Pfefferkorn CM. et al., PNAS, 2010; McGlinchey RP. et al., JBC, 2010) Moreover, at neutral pH these fibrils disassemble, supporting a highly reversible aggregation/disaggregation process that could be a way for melanosomes to recycle amyloid fibrils. Here, we investigate the conformation and aggregation state of the RPT domain in the presence of membrane mimics, since it is localized in a membranous organelle. Specifically, micelles formed from detergents like sodium dodecyl sulfate or from lipids such as lysolipid as well as phospholipid vesicles were examined. Along with circular dichroism spectroscopy, which reports on the formation of secondary structure, we exploited the sole intrinsic Trp fluorophore (W423) located at the C-terminal region as a site-specific probe of interaction. To determine the specificity of this interaction, we also produced and examined single Trp mutants at the N-terminal region. Because the melanosome is an acidic organelle, we also explored the pH dependence of interaction in detail. Finally, we carried out aggregation experiments in the presence of lipid monomers and micelles in determining their effects on fibril formation.

**2204-Plat**

**Recognition Specificity of Proteins and Biomembranes: A Computational View**

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Cell membranes, including their individual components like membrane-bound proteins and particular lipids, attract a growing attention as very perspective pharmacological targets. Rational design of new efficient and selective compounds modulating activity of biomembranes, requires atomic-scale information on their spatial structure and dynamics under different conditions. Because such details resist easy experimental characterization, important insight can be gained via computer simulations.

We present the results of structural/dynamic computational studies of membrane proteins and peptides with diverse fold, mode of membrane binding, and biological activities: antimicrobial and cell-penetrating peptides, cardiotoxins from snake venom, transmembrane domains of receptor tyrosine kinases. The computational approach combines Monte Carlo simulations in implicit membranes, molecular dynamics in full-atom lipid bilayers, and molecular hydrophobicity potential analysis. The predictive power of the method was proven via testing against high-resolution experimental data.

Despite different structure and mechanism of membrane permeation, in all cases the polypeptide-membrane recognition reveals a prominent ''self-adapting'' character. Namely, the membrane active agents employ a wide arsenal of structural/dynamic tools in order to insert into lipid bilayer and to accomplish their function. Importantly, lipid bilayer of biological membranes plays essential role in the recognition/binding events. In particular, the membrane surface reveals highly dynamic lateral heterogeneities (clusters), which differ in their packing and hydrophobic properties from the bulk lipids. Such a mosaic nature of membranes is tuned in a wide range by the chemical nature and relative content of lipids, presence of ions, etc. This makes possible mutual adaptation of the two amphiphatic systems (peptide and membrane). Such a diversity of the factors important for polepeptide-bilayer interactions assures their efficient and robust binding to cell membranes. Understanding of such effects creates a basis for rational design of new physiologically active molecules and/or artificial membranes with predefined properties.

**2205-Plat**

**Cardiolipins Binding Sites on Respiratory Chain Complexes**

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Mitochondria are the power plants of the cell. Most of the ATP used by a cell is produced by the respiratory chain located in the inner membrane of mitochondria.

It is now well accepted that the protein complexes forming the respiratory chain assemble into larger structures, the so-called supercomplexes[1]. The lack of cardiolipin (CL), a double charged phosphoslipid composing more than 10% of the mitochondrial membrane, impairs the formation of these supercomplexes[2,3] and affects their functionality in the respiratory chain[4].

To investigate the mechanism by which CLs favor the formation of supercomplexes we have simulated complexes III and IV embedded in POPC bilayers containing CLs. The use of the MARTINI coarse grained force field[5] was necessary to reach the system size and time scale necessary to this study. Most notably we found that CLs present preferential interfaces on both complexes III and IV. This led us to the hypothesis that these interfaces might play a role in the relative orientation of the complexes in the supercomplexes. This was clearly shown by the comparison of the supercomplexes formed during self-assembly simulations of a mixture of the two complexes in a lipid bilayer with and without CLs present.

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**2206-Plat**

**Cholesterol Enhances or Reduces Metarhodopsin II Formation Depending on Bilayer Thickness**

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Cholesterol is one of the most efficient modulators of G Protein-Coupled Receptor (GPCR) function. We monitored the effect of cholesterol on rhodopsin function for a set of bilayers with different hydrophobic thickness. Surprisingly, cholesterol shifts the Metarhodopsin-I (MI)/Metarhodopsin-II (MII) equilibrium toward MII for bilayers thinner than the average length of hydrophobic transmembrane helices (2.7 nm), and to MI for thicker bilayers. In previous work conducted on rod outer segment disks and model membranes, increasing cholesterol concentration always shifted the equilibrium towards MI. It was proposed that the cholesterol effect is primarily related to a tighter packing of lipid hydrocarbon chains which generates a less permissive environment for the formation of MII. To gain deeper insights into mechanisms, we followed changes in lipid-rhodopsin interaction by $^2$H NMR using deuterated lipids. It was reported by us and the Brown laboratory that an increase of bilayer hydrophobic thickness in the absence of cholesterol favors MII with a turnover to MI for bilayers that are very thick. Indeed, the cholesterol-induced shifts towards MII for thinner membranes correlated nicely with the cholesterol-induced increase of bilayer hydrophobic thickness measured by NMR suggesting that the increase in bilayer thickness by cholesterol plays a major role in controlling the energetics of the MI-MII equilibrium. Furthermore, changes in average